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(54) **METHOD OF TREATING GLYCOGEN STORAGE DISEASE TYPE III WITH HUMAN ACID  $\alpha$ -GLUCOSIDASE**

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None  
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(57) **ABSTRACT**

The present invention relates, in general, to Glycogen Storage Disease and, in particular, to a method of treating Glycogen Storage Disease-type-III and to compounds and compositions suitable for use in such a method.

**9 Claims, 5 Drawing Sheets**



Figure 1.



Figure 2. cytoplasmic glycogen forming lakes within myocyte under EM (patient MG)

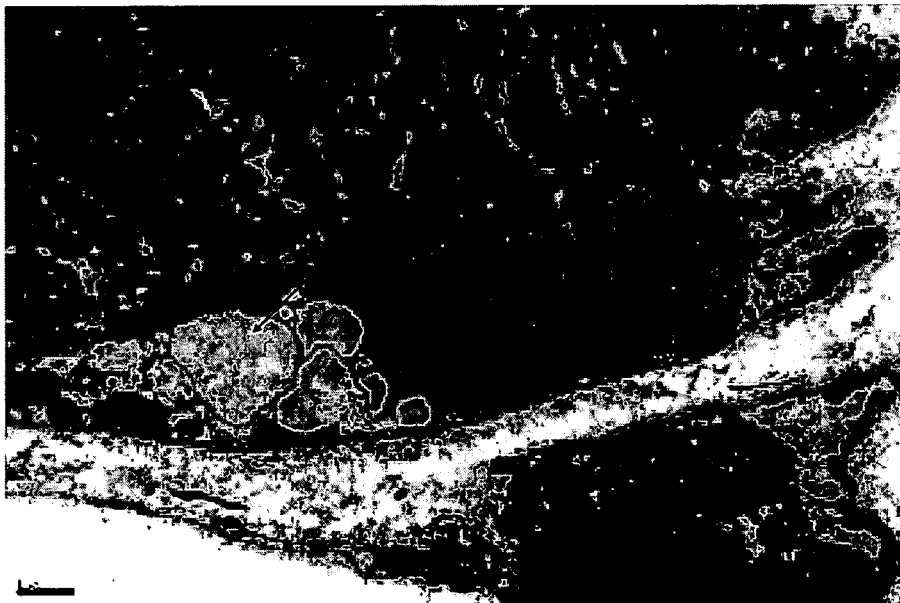


Figure 3. Lysosomal glycogen was seen within myocyte (patient MG)

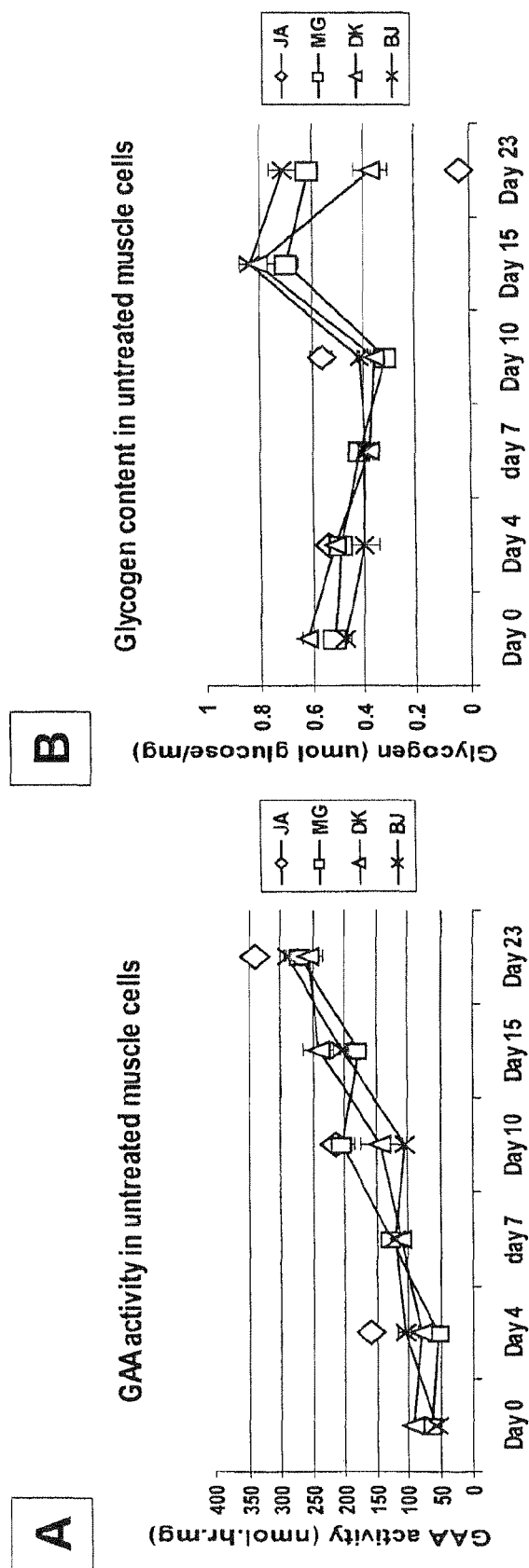


Figure 4.

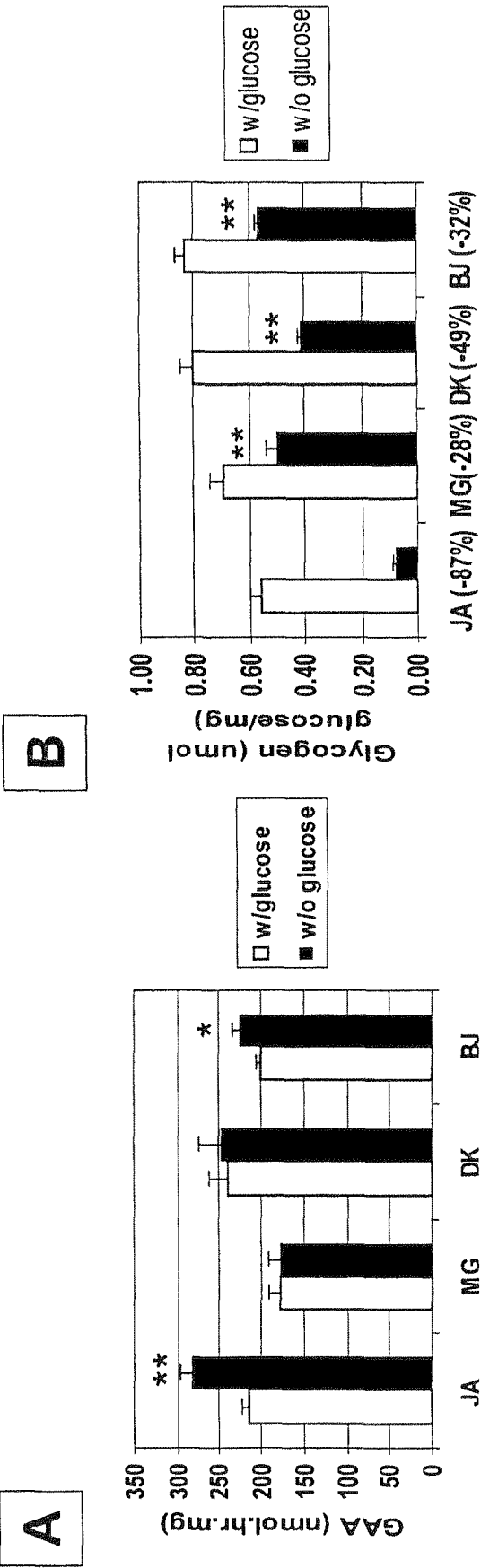


Figure 5.

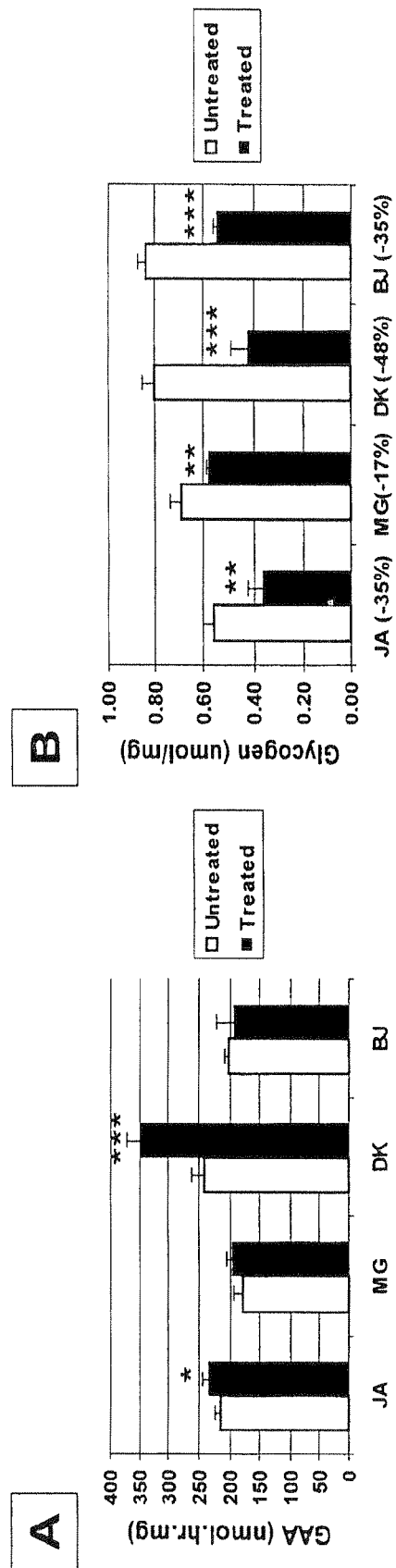


Figure 6.

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# METHOD OF TREATING GLYCOGEN STORAGE DISEASE TYPE III WITH HUMAN ACID $\alpha$ -GLUCOSIDASE

This application is the U.S. national phase of International Application No. PCT/US2009/003993, filed 8 Jul. 2009, which designated the U.S. and claims the benefit to U.S. Provisional Application No. 61/129,612, filed Jul. 8, 2008, the entire contents of each of which are incorporated herein by reference.

## TECHNICAL FIELD

The present invention relates, in general, to Glycogen Storage Disease and, in particular, to a method of treating Glycogen Storage Disease-type-III and to compounds and compositions suitable for use in such a method.

## BACKGROUND

Glycogen debranching enzyme (GDE) is a multifunctional enzyme acting as 1,4- $\alpha$ -D-glucan: 1,4- $\alpha$ -D-glucan 4- $\alpha$ -D-glycosyltransferase (E.C. 2.4.1.25) and amylo-1,6-glucosidase (E.C. 3.2.1.33) in glycogen degradation. The two activities of the debranching enzyme are believed to reside at separate sites on a single polypeptide chain with a molecular mass of 174 kDa. The structure-function domain has not been studied in detail (Chen and Burchell, Glycogen storage disease. In: *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver et al, eds., 7<sup>th</sup> Edition McGraw-Hill/ New York, pp. 935-965 (1995); Bates et al, *FEBS Lett.* 58:181-185 (1975); Gillard et al, *Biochemistry* 16:3978-3987 (1977); Chapter 71 Kishnani P S, Koeberl D, Chen Y T. *Glycogen Storage Diseases, in The Online Metabolic & Molecular Bases of Inherited Disease*, Valle D, Beaudet A L, Vogelstein B, Kinzler K W, Antonarakis S E, Ballabio A, Scriver C R, Sly W S, Childs B, Editors (2008)). The predominant form of cDNA that encodes human debrancher has a 4596 bp coding region and a 2371 bp 3' nontranslated region (Yang et al, *J. Biol. Chem.* 267:9294-9299 (1992)). Tissue specific debrancher mRNAs exist. These isoforms differ at the 5' nontranslated region and are believed to be generated by differential RNA transcription and splicing from a single debrancher gene (Bao et al, *Gene* 197:389-398 (1997)). The human gene is localized to chromosome 1p21 (Yang-Feng et al, *Genomics* 13:931-934 (1992)). The genomic structure of the human GDE gene has been determined and consists of 35 exons spanning ~85 kb of DNA.

Debranching enzyme, together with phosphorylase, is responsible for complete degradation of glycogen. Liver and muscle are the two major organs most active in glycogen metabolism. The primary function of glycogen in these organs is different. In muscle, glycogen provides a local fuel store for short-term energy consumption. In liver, it maintains glucose homeostasis.

Genetic deficiency of glycogen debranching enzyme (Glycogen Storage Disease-type III, GSD-III) causes an incomplete glycogenolysis resulting in accumulation of glycogen with abnormally short outer chain in various organs. The commonly affected organs in GSD-III are liver, skeletal muscle and heart. The disease is characterized by hepatomegaly, hypoglycemia, short stature, variable myopathy and cardiomyopathy. Patients with this disease vary remarkably, both clinically and enzymatically (Markowitz et al, *Gastroenterology* 105:1882-1885 (1993); Shen et al, *J. Clin. Invest.* 98:352-357 (1996); Telente et al, *Annals. Intern. Med.* 120: 218-226 (1994)). Most patients have disease involving both

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liver and muscle (type IIIa), some patients (~15% of all GSD-III patients) have only liver involvement (type IIIb), and, in rare cases, there is a selective loss of only one of the two GDE activities (glucosidase, (type IIIc) or transferase (type IIId)). Liver symptoms in GSD-III can improve with age and may disappear after puberty. Overt liver cirrhosis has been seen in some patients, some have developed hepatocellular carcinoma. Muscle weakness, though minimal during childhood, may become predominant in adults with onset in the third or fourth decade. These patients have slowly progressive proximal weakness and distal muscle wasting and some patients become wheelchair bound. Even within the subgroup of patients who develop myopathy/cardiomyopathy there is clinical variability. Some patients have asymptomatic cardiomyopathy, some have early symptomatic cardiomyopathy leading to death, and some have only muscle and no apparent heart involvement. An abnormal electrocardiogram (ECG) with ventricular hypertrophy is a frequent finding and does not correlate with clinical severity. Normal serum creatine kinase levels do not rule out muscle enzyme deficiency. The biochemical subtypes do not predict clinical severity.

There appears to be no correlation between the amount of debrancher protein and clinical severity (Yang et al, *Am. J. Hum. Genet.* 41:A28 (1992)). To predict accurately at initial diagnosis whether myopathy or cardiomyopathy may occur, one must determine whether debranching enzyme activity is deficient in muscle (Chen and Burchell, Glycogen storage disease. In: *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver et al, eds., 7<sup>th</sup> Edition McGraw-Hill/ New York, pp. 935-965 (1995)). It appears that muscle disease will not develop in patients with GDE activity retained in muscle (Coleman et al, *Annals of Internal Medicine* 116:896-900 (1992)).

The variable phenotype is, in part, explained by differences in tissue-specific expression of the defective enzyme. As pointed out above, in type IIIc, enzyme is deficient in both liver and muscle, in IIIb there is enzyme deficiency only in liver. Unlike phosphorylase, which has tissue-specific isoenzymes encoded by different genes, at the protein level and at the molecular level it appears that there are no tissue-specific GDE isoenzymes in different tissues. Until now, it has not been understood in GSD-III how a single GDE gene, normally expressed in all tissues, can change expression in different tissues (Chen and Burchell, Glycogen storage disease. In: *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver et al, eds., 7<sup>th</sup> Edition McGraw-Hill/New York, pp. 935-965 (1995)). Two mutations (17delAG and G6X), both located in exon 3 at amino acid codon 6, are exclusively found in the GSD-IIIb (Shen et al, *J. Clin. Invest.* 98:352-357 (1996)) suggesting that exon 3 is important in controlling tissue-specific expression of the GDE gene.

Histology of the liver in these patients is characterized by a universal distension of hepatocytes by glycogen and the presence of fibrous septa. Electron microscopy studies on muscle specimens have shown presence of accumulated glycogen beneath the sarcolemma and between myofibrils; the excess glycogen not only disperses in the cytoplasm, but is also seen in the lysosomes (Cornelio et al, *Arch. Neurol.* 41:1027-1032 (1984); Miranda et al, *Ann. Neurol.* 9:283-288 (1981)).

The detailed structural biology of GDE is not known, although several functional domains of glycogen debranching enzyme have been proposed from enzymological studies and sequence comparison to other enzymes with similar catalytic function (Yang et al, *J. Biol. Chem.* 267:9294-9299 (1992); Liu et al, *Archives of Biochemistry and Biophysics* 306:232-239 (1993); Liu et al, *Biochemistry* 34:7056-7061 (1995); Jespersen et al, *Journal of Protein Chemistry* 12(6):

791-805 (1993)). A region at the COOH-terminal of the debranching enzyme could be a candidate for glycogen binding site (Yang et al, *J. Biol. Chem.* 267:9294-9299 (1992)), and 4 regions at N-terminal half of the enzyme bear sequence homology to the catalytic sites identified or proposed in other amyolytic enzymes (Liu et al, *Archives of Biochemistry and Biophysics* 306:232-239 (1993), Jespersen et al, *Journal of Protein Chemistry* 12(6):791-805 (1993)). Aspartate at position 549 has been identified as the catalytic nucleophile in the transferase site of rabbit muscle glycogen debranching enzyme (Braun et al, *Biochemistry* 35:5458-5463 (1996)).

Currently there is no effective treatment for the disease. Hypoglycemia can be controlled by frequent meals high in carbohydrates with cornstarch supplements or nocturnal gastric drip feedings. Patients with myopathy have been given diets high in protein during the daytime plus overnight enteral infusion. In some patients, transient improvement in symptoms has been documented but there are no long-term data demonstrating that the high protein diet prevents or treats the progressive myopathy (Chen and Burchell, *Glycogen storage disease*. In: *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver et al, eds., 7<sup>th</sup> Edition McGraw-Hill/ New York, pp. 935-965 (1995)). The progressive myopathy and/or cardiomyopathy is a major cause of morbidity in adults and patients with progressive liver cirrhosis and hepatic carcinoma have been reported. While gene therapy delivery of a normal, functional gene into the diseased organ could ultimately cure the disease, an ideal gene delivery vehicle that is reliable is currently not available. There is no living animal model for this disease. Dogs affected with GSD-III have been reported (Gregory et al, *J. Vet. Intern. Med.* 21(1):40-46 (2007)), and a breeding colony is currently being established.

Enzyme replacement therapy has been effective in diseases in which the responsible enzymes/proteins exert their functions in extracellular fluids, such as adenosine deaminase deficiency, hemophilia, and  $\alpha$ 1-antitrypsin deficiency, or in a lysosomal location such as a lysosomal storage disease. Enzyme replacement has not been explored in diseases in which the defective enzyme is present in cytosol (such as the debranching enzyme in GSD-III), presumably due to the lack of an efficient and specific cellular uptake mechanism that delivers exogenous enzyme across the plasma membrane into the cytoplasm. Liposomes can fuse with plasma membrane and deliver their content, however, the use of liposomes is compromised by lack of organ-specific tropism and clearance by the reticulo-endothelial system (Mumtaz et al, *Glycobiology* 1(5):505-510 (1991)). For an effective treatment for GSD-III, the enzyme should be able to target muscle and heart as well as liver.

Cytoplasmic glycogen is normally digested by phosphorylase and debranching enzyme; excess glycogen taken up by lysosomes through autophagy can be digested by lysosomal acid  $\alpha$ -glucosidase (GAA). Deficiency of debranching enzyme activity results in massive accumulation of glycogen having abnormally short outer branches. The excess glycogen in GSD-III resides not only in the cytoplasm but also in the lysosomes (cytoplasm>lysosome) (Cornelio et al, *Arch. Neurol.* 41:1027-1032 (1984), Miranda et al, *Ann. Neurol.* 9:283-288 (1981)). This suggests that the "normal" GAA activity in GSD-III may not be sufficient to digest all the excess glycogen. GAA is a lysosomal exo 1,4- $\alpha$ -D-glucosidase that hydrolyzes both  $\alpha$ -1,4 and  $\alpha$ -1,6 linkages of glycogen and can completely digest glycogen with and without abnormally short outer branches (Onodera et al, *J. Biochem.* 116:7-11 (1994)). GAA thus acts on glycogen with abnormally short outer branches such as accumulates in GSD-III. As this gly-

cogen in GSD-III accumulates both in cytoplasm and lysosomes, providing more GAA may help to digest lysosomal glycogen and hence also cytoplasmic glycogen. It is postulated that, as the lysosomes are cleared of glycogen, glycogen from the cytoplasm shuffles into them thus decreasing the total amount of accumulated glycogen in GSD-III patients.

Deficiency of GAA causes Pompe disease (type II glycogen storage disease), a fatal metabolic myopathy with accumulation of glycogen in lysosome and cytoplasm (lysosome>cytoplasm) (Hirschhorn, *Glycogen Storage Disease Type II: Acid  $\alpha$ -glucosidase (Acid Maltase) Deficiency*. In: *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver et al, eds., 7<sup>th</sup> Edition McGraw-Hill/New York, pp. 2443-2464 (1995)). Enzyme replacement therapy with mannose-6-Phosphate (man-6-P)-rich precursor recombinant human GAA results in efficient man-6-P receptor mediated endocytosis of the enzyme followed by reduction of both lysosomal and cytoplasmic glycogen in fibroblasts (Van Hove et al, *Proc. Natl. Acad. Sci.* 93:65-70 (1996)). In vivo, this enzyme targets heart and muscle as well as liver and spleen following intravenous injection in animals and in human Pompe patients. The rapid clearance of glycogen in Pompe fibroblasts when cultured in glucose-free medium suggests a ready mobilization of glycogen from both lysosomal and cytoplasmic compartments (Van Hove et al, *Proc. Natl. Acad. Sci.* 93:65-70 (1996), DiMauro et al, *Pediatr. Res.* 7:739-744 (1973)). It is contemplated that cytoplasmic glycogen continuously shuffles through lysosomes by autophagy for degradation.

The present invention results, at least in part, from the realization that administered GAA can reduce lysosomal glycogen in GSD-III patients and ultimately also reduce cytoplasmic glycogen. Some of the administered GAA may also go directly into the cytosol and reduce the glycogen. The invention provides a method of treating GSD-III (as well as GSD-IV, -VI, -IX, XI and cardiac glycogenosis due to AMP-activated protein kinase gamma subunit 2 deficiency) based on the use of GAA.

## SUMMARY OF THE INVENTION

The present invention relates generally to GSD. More specifically, the invention relates to methods of treating GSD-III and to compounds and compositions suitable for use in such methods.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. The light microscopic appearance of the skeletal muscle biopsy from a GSD-IIIa patient. The purple (dark areas) staining glycogen is present as cytoplasmic lakes within myocytes. The glycogen is not membrane bound within lysosomes but is free flowing within the cytoplasm.

FIG. 2. Cytoplasmic glycogen forming lakes within myocyte under EM in a patient with GSD-III.

FIG. 3. Lysosomal glycogen was seen within myocyte of a patient with GSD-III.

FIGS. 4A and 4B. Pattern of glycogen accumulation in muscle cells derived from a normal subject (JA) and 3 GSD-III patients (MG, DK and BJ). Duplicate cultures were harvested at times indicated. Cellular GAA activity (FIG. 4A) and glycogen content (FIG. 4B) were determined in duplicates for each culture (mean $\pm$ SD).

FIGS. 5A and 5B. Glycogen depletion in untreated muscle cells from a normal subject (JA) and 3 GSD-III patients (MG, DK and BJ) by glucose starvation. GAA activity (FIG. 5A) and glycogen content (FIG. 5B) were analyzed in GSD-III



muscle cells after 48-hour culturing (from Day 13 to Day 15) in differentiation medium with (w/) glucose or without (w/o) glucose. (\*,  $p$  value $<0.01$ ; \*\*,  $p$  $<0.001$ ).

FIGS. 6A and 6B. rhGAA uptake and glycogen reduction in GSD-III muscle cells from patients MG, DK and BJ after 48-hour treatment (from Day 13 to Day 15) with 100  $\mu$ g of rhGAA. Muscle cells from normal subject (JA) were treated from Day 7 to Day 10. (\*,  $p$  value $<0.05$ ; \*\*,  $p$  $<0.005$ ; \*\*\*,  $p$  $<0.001$ ).

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of treating GSD, particularly GSD-III, by administering GAA to an individual suffering from the disease. The invention also relates to the use of the enzyme, GAA, in the manufacture of a medicament for the treatment of GSD (e.g., GSD-III). As described herein, patients suffering from, for example, GSD-III can be treated by administering GAA on, for example, a regular basis. Patients thus treated can be expected to demonstrate improvement of hypoglycemia, hepatomegaly, hepatic function, cardiac status, and/or muscular strength, as well as a reduction of tissue glycogen levels.

The invention makes possible the treatment of GSD-III, including GSD-type IIIa, type IIIb, type IIIc or type IIId. The invention also makes possible the treatment of other forms of GSD, including, but not limited to, GSD-IV, -VI, -IX, XI and cardiac glycogenosis due to AMP-activated protein kinase gamma subunit 2 deficiency.

The terms, "treat" and "treatment," as used herein, refer to amelioration of one or more symptoms associated with the disease, prevention or delay of the onset of one or more symptoms of the disease, and/or lessening of the severity or frequency of one or more symptoms of the disease. For example, treatment can refer to improvement of hypoglycemia, growth retardation, hepatomegaly, and hepatic function (e.g., reduction of SGOT, SGPT); cardiac status (e.g., reduction, amelioration or prevention of the progressive cardiomyopathy, arrhythmia and other cardiac manifestations that can be found, for example, in GSD-III), myopathy (e.g., exercise tolerance), reduction of glycogen levels in tissue (e.g., liver and muscle) of the individual affected by the disease, or any combination of these effects. Further, the treatment may prevent long term complications such as liver cirrhosis, hepatocellular carcinoma due to clearance of glycogen with an abnormal structure. In one preferred embodiment, treatment includes improvement of liver symptoms, particularly, in reduction or prevention of GSD (e.g., GSD-III)-associated hypoglycemia, hepatomegaly and abnormal liver function. The terms, "improve," "prevent" or "reduce," as used herein, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A control individual is an individual afflicted with the same form of the disease (e.g., GSD-III) as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

The individual being treated can be an individual (infant, child, adolescent, or adult human) having GSD-III. The individual can have residual GDE activity, or no measurable activity. In another preferred embodiment, the individual is an individual who has been recently diagnosed with the disease. Early treatment (treatment commencing as soon as possible

after diagnosis) is important to minimize the effects of the disease and to maximize the benefits of treatment.

While the invention is described in detail with reference to GSD-III, the methods described herein can also be used to treat individuals suffering from other GSDs, including, but not limited to, GSD-IV, -VI, IX and -XI. The methods described herein can also be used in the treatment of individuals suffering from cardiac glycogenosis due to AMP-activated protein kinase gamma subunit 2 deficiency.

In the methods of the invention, GAA (preferably, human GAA) is administered to the individual. The GAA is in a form that, when administered, targets tissues such as the tissues affected by the disease (e.g., liver, heart or muscle). In one preferred embodiment, human GAA is administered in its precursor form, as the precursor contains motifs that allow efficient receptor-mediated uptake of GAA. Alternatively, a mature form of human GAA that has been modified to contain motifs to allow efficient uptake of GAA into cells, can be administered. In a particularly preferred embodiment, the GAA is the precursor form of recombinant human GAA.

GAA is obtainable from a variety of sources. In a particularly preferred embodiment, recombinant human acid  $\alpha$ -glucosidase (rhGAA) produced in Chinese hamster ovary (CHO) cell cultures is used (see, e.g., Fuller, M. et al., *Eur. J. Biochem.* 234:903 909 (1995); Van Hove, J. L. K. et al., *Proc. Natl. Acad. Sci. USA* 93:65 70 (1996) and U.S. Pat. No. 7,056,712). Production of GAA in CHO cells yields a product having glycosylation that allows significant and efficient uptake of GAA in tissues such as heart and muscle. MYOZYME (alglucosidase alpha) Genzyme Corp.), or other recombinant human GAA, can be used in accordance with the invention.

The GAA has a specific enzyme activity in the range of about 1.0-8.0  $\mu$ mol/min/mg protein, preferably in the range of about 4.0-8.0  $\mu$ mol/min/mg protein. In one preferred embodiment, the GAA has a specific enzyme activity of at least about 1.0  $\mu$ mol/min/mg protein; more preferably, a specific enzyme activity of at least about 4.0  $\mu$ mol/min/mg protein; even more preferably, a specific enzyme activity of at least about 6.0  $\mu$ mol/min/mg protein; and still more preferably, a specific enzyme activity of at least about 8.0  $\mu$ mol/min/mg protein.

GAA can be administered alone, or in compositions or medicaments comprising the GAA, as described herein. The compositions can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, sugars such as mannitol, sucrose, or others, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. In a preferred embodiment, a water-soluble carrier suitable for intravenous administration is used.

The composition or medicament, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release

formulation, or powder. The composition can also be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate, etc.

The composition or medicament can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, in a preferred embodiment, a composition for intravenous administration typically is a solution in sterile isotonic aqueous buffer. Where necessary, the composition can also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The GAA can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

GAA (or composition or medicament containing GAA) is administered by an appropriate route. In one embodiment, the GAA is administered intravenously. In other embodiments, GAA is administered by direct administration to a target tissue, such as heart or muscle (e.g., intramuscular). In yet another embodiment, GAA is administered orally. More than one route can be used concurrently, if desired.

GAA (or composition or medicament containing GAA) can be administered alone, or in conjunction with other agents, such as antihistamines (e.g., diphenhydramine) or immunosuppressants or other immunotherapeutic agents which counteract anti-GAA antibodies. Possible immunomodulation strategies include preventive tolerance induction either with initiation of therapy, or tolerance modulation after the development of inhibitory antibodies. The term, "in conjunction with," indicates that the agent is administered at about the same time as the GAA (or composition containing GAA). For example, the agent can be mixed into a composition containing GAA, and thereby administered contemporaneously with the GAA; alternatively, the agent can be administered contemporaneously, without mixing (e.g., by "piggybacking" delivery of the agent on the intravenous line by which the GAA is also administered, or vice versa). In another example, the agent can be administered separately (e.g., not admixed) but within a short time frame (e.g., within 24 hours) of administration of the GAA. In one embodiment, GAA (or composition containing GAA) is administered in conjunction with an immunosuppressive or immunotherapeutic regimen designed to reduce amounts of, or prevent production of, anti-GAA antibodies. For example, a protocol similar to those used in hemophilia patients (Nilsson, I. M. et al., *N. Engl. J. Med.* 318:947-50 (1988)) can be used to reduce anti-GAA antibodies. In a particularly preferred embodiment, the immunosuppressive or immunotherapeutic regimen is begun prior to the first administration of GAA, in order

to minimize the possibility of production of anti-GAA antibodies. As an example, use of Rituximab, which eliminates mature B cells expressing CD20, methotrexate, which acts on both B and T cells, or different combinations of such agents is possible (Mendelson et al, *N. Engl. J. Med.* 360(2):194-195 (2009)).

GAA (or composition or medicament containing GAA) is administered in a therapeutically effective amount (i.e., a dosage amount that, when administered, for example, at regular intervals, is sufficient to treat the disease, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease, as described above). The amount that will be therapeutically effective in the treatment of the disease will depend on the nature and extent of the disease's effects, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed can also depend on the route of administration, and the seriousness of the disease, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. In a preferred embodiment, the therapeutically effective amount is less than about 40 mg enzyme/kg body weight of the individual, preferably in the range of about 1-40 mg enzyme/kg body weight, and even more preferably about 20 mg enzyme/kg body weight or about 10 mg enzyme/kg body weight. The effective dose for a particular individual can be varied (e.g., increased or decreased) over time, depending on the needs of the individual. For example, in times of physical illness or stress, or if anti-GAA antibodies become present or increase, or if disease symptoms worsen, the amount can be increased.

The therapeutically effective amount of GAA (or composition or medicament containing GAA) can be administered at regular intervals, depending on the nature and extent of the disease's effects, and on an ongoing basis. Administration at a "regular interval," as used herein, indicates that the therapeutically effective amount is administered periodically (as distinguished from a one-time dose). The interval can be determined by standard clinical techniques. In preferred embodiments, GAA is administered monthly, bimonthly, weekly, twice weekly, or daily. The administration interval for a single individual need not be a fixed interval but can be varied over time, depending on the needs of the individual. For example, in times of physical illness or stress, if anti-GAA antibodies become present or increase, or if disease symptoms worsen, the interval between doses can be decreased.

In one preferred embodiment, a therapeutically effective amount of 20 mg enzyme/kg body weight is administered bi-monthly. In another preferred embodiment, a therapeutically effective amount of 10 mg enzyme/kg body weight is administered weekly or 5 mg enzyme/kg body weight is administered twice weekly.

The invention additionally pertains to a pharmaceutical composition comprising human GAA, as described herein, in a container (e.g., a vial, bottle, bag for intravenous administration, syringe, etc.) with a label containing instructions for administration of the composition for treatment of GSD-III, such as by the methods described herein.

Certain aspects of the invention are described in greater detail in the non-limiting Examples that follow. (See also U.S. Pat. No. 7,056,712.)

#### Example 1

GAA is a lysosomal exo 1,4- $\alpha$ -D-glucosidase that hydrolyzes both  $\alpha$ -1,4 and,  $\alpha$ -1,6 linkage of glycogen. A highly

efficient system has been developed for producing human GAA which targets heart and muscle and corrects glycogen accumulation in patients with Pompe disease. The excess glycogen in GSD-III resides not only in the cytoplasm but also in the lysosome (Cornelio et al, *Arch. Neurol.* 41:1027-1032 (1984), Miranda et al, *Ann. Neurol.* 9:283-288 (1981)). This suggests that excess cytoplasmic glycogen is shuffled more effectively into the lysosomes than can be cleared by the "normal" GAA activity in GSD-III cells. It is hypothesized that cytoplasmic glycogen continuously shuffles through lysosomes by autophagy for degradation, and that the administered GAA can reduce lysosomal glycogen in GSD-III and ultimately also reduce cytoplasmic glycogen.

#### Source of the Enzyme

High GAA producing CHO cells can be grown in expanded culture and recombinant enzyme purified from the medium. Milligram quantities of purified GAA is available weekly as part of an ongoing project on the development of enzyme replacement therapy for Pompe disease. MYOZYME (Genzyme Corp.), or other rhGAA (preferably CHO-produced), is suitable for use in the instant invention.

#### Tissue Source

Muscle samples can be obtained from patients with GSD-IIIa whose disease has been previously diagnosed by demonstrating debrancher activity deficiency in both liver and muscle. Needle muscle biopsy from patients with the diagnosis can be performed. It is expected that at least 3 patients with a confirmed diagnosis of GSD-IIIa will be studied. The needle muscle biopsy can obtain 50-70 mg of tissues sufficient for analysis. The procedure is less invasive than an open biopsy. Currently, skin fibroblasts from 9 GSD-IIIa patients are available. An IRB approved protocol is available that makes it possible to obtain muscle and skin tissue from patients with GSD III.

#### Cell Culture

Cultured skin fibroblasts and muscle cells can be used to test the feasibility of using GAA to treat GSD-III. The focus will be particularly on IIIa patients who have, in addition to hepatomegaly, progressive myopathy (increasing muscle weakness by muscle strength testing in 6 months to 1 year follow-up and/or clinical complaints of a decrease in muscle strength) and cardiomyopathy (increase in left ventricular mass in a 6-12 month period on follow-up), and also patients with progressive liver disease. Deficiency of debranching enzyme and accumulation of glycogen are present in skin fibroblasts and muscle cells grown in culture from GSD-III patients (Miranda et al, *Ann. Neurol.* 9:283-288 (1981), DiMauro et al, *Pediatr. Res.* 7:739-744 (1973), Yang et al, *Am. J. Hum. Genet.* 47:735-739 (1990), Brown, *Diagnosis of glycogen storage disease*, In: Wapnir Pa., (ed.), *Congenital Metabolic Disease Diagnosis and Treatment*, Dekker, New York, pp. 227-250 (1985)). Cultured muscle cells also reflect muscle biopsy findings in that both cytoplasmic and lysosomal glycogen are observed (Cornelio et al, *Arch. Neurol.* 41:1027-1032 (1984), Miranda et al, *Ann. Neurol.* 9:283-288 (1981)). The skin fibroblasts and muscle cultures can, therefore, be used to improve understanding of the pathogenesis of the disease and to evaluate approaches to therapy. Muscle cultures can be established from muscle biopsy samples using a protocol similar to the isolation of quail myoblasts (Konigsberg, *Methods in Enzymology* 45:511-527 (1979)). Replating of the primary cultures can be performed to select against fibroblasts. If the biopsy sample is too small, a more efficient myoblasts isolation method using the fluorescence-activated cell sorter can be performed (Webster, *Experimental Cell Research* 174:252-265 (1988)). Myoblasts can be allowed to differentiate to myotubes by changing the medium to 2%

horse serum. Muscle cells in the appropriate stage will be used for conduct of the experiments.

#### Effect on Glycogen with GAA Treatment

Glycogen concentrations in skin fibroblasts vary with cell confluence and passage number (DiMauro et al, *Pediatr. Res.* 7:739-744 (1973)). Experiments can be performed at near confluence, and each patient can serve as his/her own control by concurrent duplicate testing with and without GAA treatment. Dose response (from 500 to 5000 nmol/hr/ml of GAA) and time course (1 to 10 days) can be tested for the effect of glycogen reduction. Glycogen content can be measured in total cell homogenates and also individually in cytosol and crude lysosomal fractions (van der Ploeg et al, *J. Neurol. Sci.* 79:327-336 (1987)). Electromicroscopic examination can be performed for evidence of clearance in both cytoplasm and lysosomes.

To avoid obscuring of the results by the continuous glycogen synthesis that occurs in cells cultured in the presence of glucose in the medium, cells can be shifted to glucose-free medium 24 hours after GAA treatment. Deprivation of glucose resulted in greater than 80% drop of glycogen in the normal cells, but only 30% reduction in GSD-III cells (Yang et al, *Am. J. Hum. Genet.* 47:735-739 (1990), Brown, *Diagnosis of glycogen storage disease*, In: Wapnir P A, (ed.), *Congenital Metabolic Disease Diagnosis and Treatment*, Dekker, New York, pp. 227-250 (1985)). Thus sufficiently high levels of glycogen persist which allow accurate evaluation of GAA treatment. The persistent glycogen in GSD-III cells have short outer brancher which can be assessed using glucose-1-phosphate formed from endogenous polysaccharide by phosphorylase (Yang et al, *Am. J. Hum. Genet.* 47:735-739 (1990)).

Example 2 below includes a description of studies that have been undertaken.

#### Example 2

Primary human skeletal muscle cultures from GSD-IIIa patients have been established as an in vitro model to evaluate efficacy of rhGAA for treatment of GSD-III. Myoblasts were isolated from three GSD-IIIa patients and one healthy volunteer. Histopathology of these muscle biopsies was examined by light and electronic microscopy (EM) to confirm abnormal glycogen accumulation in these GSD-IIIa patients. The light microscopic appearance of the skeletal muscle biopsies from GSD-IIIa patients showed abundant glycogen accumulation in cytoplasmic pools (FIG. 1). Under EM, the vast majority of the glycogen was found free in the cytoplasm along with small amount of membrane-bound glycogen (FIGS. 2 and 3).

Differentiation of myoblasts into mature myotubes (myogenesis) was induced by incubation of the muscle cells in low-serum differentiation

medium (low-glucose DMEM (GIBCO) containing 2% Hyclone H1 horse serum (Sigma), 0.5 mg/ml Fetuin, 0.5 mg/ml BSA, 0.025 mg/ml gentamycin and 0.125 µg/ml Amphotericin B (Clonetics). Glycogen was stored in fully differentiated GSD-III muscle cells when sufficient glucose was supplied in the medium (FIG. 4). Incomplete glycogenolysis was seen in GSD-III cells, but not in normal control cells after 48-hour glucose starvation (FIG. 5), indicating lack of debranching enzyme activity in the GSD-III patients. Fully differentiated GSD-III myotubes were treated for 48 hours by adding 100 µg of rhGAA (i.e., MYOZYME (Genzyme)) into the culture medium. GAA enzyme activity and glycogen content were analyzed biochemically and histologically in these cells. Treatment with rhGAA significantly reduced glycogen level by 48%, 35% and 17%, respectively, in the three

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GSD-IIIa patient muscle cells (FIG. 6). These data suggest the role of GAA in glycogen clearance in conditions where the primary defect results in cytoplasmic glycogen accumulation.

What is claimed is:

1. A method of treating glycogen storage disease type III comprising administering to a human in need of such treatment an amount of human acid  $\alpha$ -glucosidase at a regular interval sufficient to effect said treatment.

2. The method of claim 1, wherein the amount of the human acid  $\alpha$ -glucosidase administered at the regular interval is less than about 40 mg of acid  $\alpha$ -glucosidase per kilogram of body weight of the individual.

3. The method of claim 1, wherein the human acid  $\alpha$ -glucosidase is recombinant human acid  $\alpha$ -glucosidase or a precursor of recombinant human acid  $\alpha$ -glucosidase.

4. The method of claim 3, wherein the recombinant human acid  $\alpha$ -glucosidase is produced in Chinese hamster ovary cells.

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5. The method of claim 1, wherein the regular interval is monthly, bimonthly, weekly, twice weekly or daily.

6. The method of claim 1, wherein the human acid  $\alpha$ -glucosidase is administered intravenously.

7. The method of claim 1, wherein the human acid  $\alpha$ -glucosidase is administered in conjunction with, or subsequent to, administration of an immunosuppressant.

8. The method of claim 1, wherein the human acid  $\alpha$ -glucosidase is administered intramuscularly, intrathecally or intraventricularly.

9. A method of treating an individual who has been diagnosed as having glycogen storage disease type III comprising administering by injection to said individual following diagnosis of glycogen storage disease type III, an amount of human acid  $\alpha$ -glucosidase at a regular interval sufficient to effect said treatment.

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